

PCT

WORLD INTELLECTUAL PROP
International I



INTERNATIONAL APPLICATION PUBLISHED UNDE

WO 9607434A1

<p>(51) International Patent Classification 6 : A61K 49/00, A61M 31/00, A61F 2/30, A61K 9/00</p>	<p>A1</p>	<p>(11) International Publication Number: WO 96/07434 (43) International Publication Date: 14 March 1996 (14.03.96)</p>
<p>(21) International Application Number: PCT/GB95/02109 (22) International Filing Date: 6 September 1995 (06.09.95) (30) Priority Data: 9417941.3 6 September 1994 (06.09.94) GB (71) Applicant (for GB only): MARSDEN, John, Christopher [GB/GB]; Frank B. Dehn & Co., Imperial House, 15-19 Kingsway, London WC2B 6UZ (GB). (71) Applicant (for all designated States except US): NYCOMED IMAGING AS [NO/NO]; Nycoveien 2, P.O. Box 4220 Torshov, N-0401 Oslo 4 (NO). (72) Inventors; and (75) Inventors/Applicants (for US only): KLAIVENESS, Jo [NO/NO]; Midtåsen 5, N-1166 Oslo (NO). RONGVED, Pål [NO/NO]; Hovdens vei 11, N-1450 Nesoddtangen (NO). STRANDE, Per [NO/NO]; Nordengveien 78A, N-0755 Oslo (NO). (74) Agent: MARSDEN, John, Christopher, Frank B. Dehn & Co., Imperial House, 15-19 Kingsway, London WC2B 6UZ (GB).</p>		<p>(81) Designated States: AM, AU, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, RO, RU, SD, SG, SI, SK, TJ, TM, TT, UA, UG, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ, UG). Published With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</p>
<p>(54) Title: IMPROVEMENTS IN OR RELATING TO CONTRAST AGENTS</p> <p>(57) Abstract</p> <p>Contrast agents comprising gas-containing polymer microparti- cles and/or microballoons in which the polymer is a biodegradable polymer comprising repeating units of formula (II) (where a is an integer of 9-19 and b is an integer of 1-8) may be used in diagnostic applications such as ultrasound and MR imaging. The polymers are novel and are also claimed <i>per se</i>.</p> <div style="text-align: center;"> $\begin{array}{c} \text{CH}_3 \\ \\ -\text{O}-(\text{CH}_2)_a-\text{CO}-\text{O}-\text{CH}-\text{O}-\text{CO}-(\text{CH}_2)_a-\text{O}-\text{CO}-(\text{CH}_2)_b-\text{CO}- \end{array} \quad (\text{II})$ </div>		

FOR THE PURPOSES OF INFORMATION ONLY

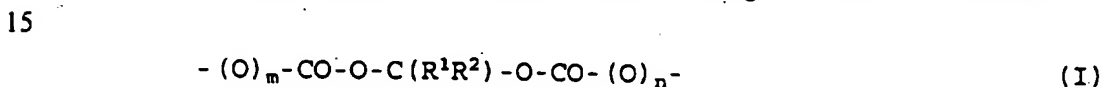
Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

IMPROVEMENTS IN OR RELATING TO CONTRAST AGENTS

5 This invention relates to novel contrast agents, more particularly to new gas-containing polymer-based contrast agents of use in diagnostic imaging, and to the novel polymer components thereof.

10 Published International Patent Application No. WO 93/17718, the contents of which are incorporated herein by reference, discloses contrast agents comprising gas-containing or gas-generating polymer microparticles and/or microballoons characterised in that the polymer is a biodegradable polymer containing units of formula



20 (wherein R^1 and R^2 each represent a hydrogen atom or a carbon-attached monovalent organic group or R^1 and R^2 together form a carbon-attached divalent organic group, and m and n are each zero or 1). Such contrast agents, which may be used in diagnostic applications such as ultrasound and MR imaging, exhibit good storage stability coupled with good stability and contrast effect in vivo following administration, often for several passages of circulation. The contrast agents are, however, thereafter readily biodegradable in vivo by virtue of the susceptibility of the units of formula (I) to degradation by common esterase enzymes.

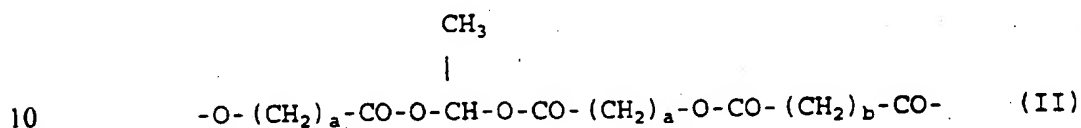
30 The present invention concerns contrast agents which fall within the overall scope of the above-mentioned WO 93/17718 but which are not specifically disclosed thereby. This novel class of contrast agents is particularly advantageous by virtue of the agents' excellent stability and contrast effect in vivo and the fact that the agents degrade in the body to products which are well-tolerated and in most cases are

35

- 2 -

endogenous.

According to one aspect of the present invention there are provided contrast agents comprising gas-containing polymer microparticles and/or microballoons characterised in that the polymer is a biodegradable
 5 polymer comprising repeating units of formula (II)



(where a represents an integer in the range 9-19, e.g. 13-17, and b represents an integer in the range 1-8, e.g. 3-6). Such contrast agents have been found to
 15 exhibit very sharp ultrasound contrast effects in animal tests, for example providing both myocardial contrast enhancement in dogs in all parts of the ventricular wall and excellent contrast enhancement of the kidney. Echogenicity may also be retained following uptake of
 20 the contrast agents by the reticuloendothelial system, permitting use as a macrophage imaging agent.

The contrast agents of the invention exhibit excellent storage stability, for example maintaining their echogenicity in an aqueous suspension for eight
 25 weeks at 25°C. They are, however, rapidly degraded and eliminated from the body following administration, e.g. having a half life of 1-2 days in the liver.

It will be appreciated that the principal in vivo degradation products of polymers comprising repeating
 30 units of formula (II) will be ω -hydroxyacids of formula $\text{HO} \cdot (\text{CH}_2)_a \cdot \text{COOH}$ (where a is as hereinbefore defined), diacids of formula $\text{HOOC} \cdot (\text{CH}_2)_b \cdot \text{COOH}$ (where b is as hereinbefore defined) and acetaldehyde. Acetaldehyde is an endogenous substance which will be oxidised in vivo
 35 to acetic acid, as in the metabolism of ethanol. The integers a and b may advantageously be chosen so as to generate endogenous ω -hydroxyacids and diacids; thus,

for example, polymers in which $a=15$ and $b=4$ will degrade to yield 16-hydroxypalmitic acid and adipic acid, both of which are endogenous.

5 The contrast agents of the invention may be used in a variety of diagnostic imaging techniques, including ultrasound, MR and X-ray imaging. Their use in diagnostic ultrasonic imaging and in MR imaging, e.g. as susceptibility contrast agents, constitute preferred features of the invention.

10 Any biocompatible gas may be employed in the contrast agents of the invention, for example air, nitrogen, oxygen, hydrogen, nitrous oxide, carbon dioxide, helium, argon, sulphur hexafluoride, low molecular weight optionally fluorinated hydrocarbons
15 such as methane, acetylene, carbon tetrafluoride and other perfluoroalkanes such as perfluoropropane, perfluorobutane and perfluoropentane, and mixtures of any of the foregoing. The gas may be free within the microbubble or may be trapped or entrained within a
20 containing substance. The term "gas" as used herein includes any substances in gaseous (including vapour) form at 37°C.

For ultrasonic applications such as
echocardiography, in order to permit free passage
25 through the pulmonary system and to achieve resonance with the preferred imaging frequency of about 0.1-15 MHz, it may be convenient to employ microbubbles having an average size of 0.1-10 μm , e.g. 1-7 μm . Substantially larger bubbles, e.g. with average sizes of
30 up to 500 μm , may however be useful in other applications, for example gastrointestinal imaging or investigations of the uterus or Fallopian tubes.

The contrast agents of the invention may incorporate additives such as emulsifying agents,
35 coating agents, plasticisers, bulking agents, cryoprotectants and/or antioxidants, for example to modify their stability, dispersibility, aggregation

tendencies, biological properties etc., or to modify the flexibility and/or polarity of the membrane.

Representative emulsifying agents include fatty acids (e.g. straight chain saturated or unsaturated fatty acids, for example containing 10-20 carbon atoms) and carbohydrate and triglyceride esters thereof; proteins such as gelatin or, more preferably, human serum albumin; phospholipids, e.g. lecithin; polysaccharides such as starch, modified (e.g. lipophilised) starch or gum arabic; and surface active polymers such as polyvinyl alcohols, polyethylene glycols and block copolymers (including extended polymers), for example poly(oxyethylene)-poly(oxypropylene)-poly(oxyethylene) block copolymers such as Pluronics.

Where block copolymer (including extended polymer) surfactants are employed, these may contain biodegradable linkages of formula (I) as hereinbefore defined, for example in which R^1 and R^2 (when other than hydrogen) may each represent a carbon-attached hydrocarbyl or heterocyclic group, for example having up to 20 carbon atoms, e.g. an aliphatic group such as an alkyl or alkenyl group (preferably having up to 10 carbon atoms), a cycloalkyl group (preferably having up to 10 carbon atoms), an araliphatic group such as an aralkyl group (preferably having up to 20 carbon atoms), an aryl group (preferably having up to 20 carbon atoms) or a heterocyclic group having up to 20 carbon atoms and one or more heteroatoms selected from O, S and N. Such a hydrocarbyl or heterocyclic grouping may carry one or more functional groups such as halogen atoms or groups of the formulae $-NR^3R^4$, $-CONR^3R^4$, $-OR^5$, $-SR^5$ and $-COOR^6$, where R^3 and R^4 are each hydrogen atoms, acyl groups or hydrocarbyl groups as defined for R^1 and R^2 ; R^5 is a hydrogen atom, an acyl group or a group as defined for R^1 or R^2 ; and R^6 is a hydrogen atom or a group as defined for R^1 or R^2 . Where R^1 and R^2 represent a divalent

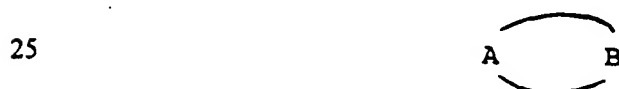
grouping this may, for example, be an alkylidene, alkenylidene, alkylene or alkenylene group (preferably having up to 10 carbon atoms), which may carry one or more functional groups as defined above.

5 The presence in such block copolymers of units of formula (I) in which R^1 and R^2 are selected from hydrogen atoms and methyl groups, e.g. in which R^1 represents a hydrogen atom and R^2 represents a methyl group, may be advantageous; it may also be advantageous to select
10 units in which m and n are zero.

 The block copolymer surfactant may comprise two or more blocks of differing lyophilicity, for example in linear di-block, tri-block or multi-block arrays, e.g. of the type A-B, A-B-A, B-A-B or A-B-A-B-A where A and B
15 are polymer blocks of differing lyophilicity, preferably being hydrophilic and hydrophobic blocks respectively. Branched structures, e.g. of the type



and macrocyclic structures, e.g. of the type



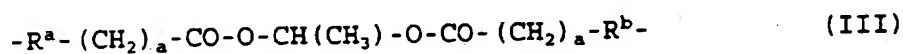
may also be employed.

 Hydrophilic blocks in such block copolymer surfactants may, for example, be derived from polymers
30 such as polysaccharides, polyalcohols (e.g. polyvinyl alcohol), polyvinylpyrrolidones, polyethylene glycols and poly(amino acids). Polymers such as polyorthoesters, polyacetals, polyanhydrides, polyglycolic acids, poly(meth)acrylic acids and
35 derivatives such as esters thereof, substituted as necessary by hydrophilic groups, may also be useful. The hydrophilic blocks may advantageously consist

essentially of polyethylene glycol units.

Hydrophobic blocks in such block copolymer surfactants may, for example, be derived from oil-soluble condensation, ionic and free-radical generated polymers, for example poly(meth)acrylate esters, polyorthoesters, vinylic and styrenic polymers, polyacetals, polyanhydrides, polyglycolic acids and ethers and esters thereof, and polylactic acid/polyglycolic acid copolymers; such polymers may, for example, incorporate or be substituted with hydrophobic groups such as alkyl, aralkyl or aryl groups to increase their hydrophobicity. The hydrophobic blocks may advantageously comprise a polyester chain containing one or more long chain aliphatic groups (e.g. C₁₀₋₂₀ polymethylene groups) linked by and/or incorporating units of formula (I). Such hydrophobic blocks may be oligomeric or quasi-polymeric, as in extended polymers, and as such may include monomeric groups, which may for example exhibit polymer characteristics (e.g. as a result of the presence of long chain units) while not strictly possessing a definable repeating unit.

One preferred class of block copolymer surfactants ~~thus comprises copolymers containing polyethylene glycol units as the hydrophilic blocks and units of formula~~



(where a is as hereinbefore defined and R^a and R^b each represent valence bonds or linking groups such as carbonyl groups or diacid residues of formula -O-CO-(CH₂)_b-CO-O where b is as hereinbefore defined) in the hydrophobic part, as an extending moiety or as a moiety in an oligomeric or polymeric block.

Other preferred classes of surfactants include fatty acid acylated polyethylene glycols such as MYRJ s and extended polymers comprising a methoxy-terminated polyethylene glycol hydrophilic block acylated with a

hydrophobic moiety comprising a chain of two or more fatty acids, for example an acyloxyacyl group such as 16-hexadecanoyloxyhexadecanoyl.

5 Representative bulking agents and cryoprotectants include alcohols, for example aliphatic alcohols such as t-butanol, polyols such as glycerol, sugars such as sucrose, mannitol, trehalose or cyclodextrins, and polyglycols such as polyethylene glycol.

10 Representative preservatives include antioxidants. The contrast agents of the invention may be prepared in a number of ways, e.g. as described in WO 93/17718, for example by incorporation of a gas into a biodegradable polymer comprising repeating units of formula (II) so as to form polymer microparticles and/or
15 microballoons.

One useful method corresponds to the interfacial deposition techniques described in EP-A-0398935 and EP-A-0458745 and comprises dissolving or suspending the polymer in a water-immiscible organic solvent,
20 emulsifying (e.g. by high speed stirring or high shear mixing) the resulting solution or suspension in an aqueous phase, preferably in the presence of a surfactant to stabilise the resulting oil-in-water emulsion, and subsequently removing at least the organic
25 phase, preferably both phases (e.g. by evaporation or lyophilisation, preferably under an atmosphere of the gas which is to be incorporated, e.g. under reduced pressure) whereby the polymer forms a membrane at the interface between the aqueous and organic phases.

30 Organic solvents useful in such processes include aliphatic, cycloaliphatic and araliphatic hydrocarbons, e.g. containing up to 10 carbon atoms, for example n-octane, cyclooctane, cyclohexane, a dimethylcyclohexane, ethylcyclohexane, a methylheptane, an ethylhexane,
35 toluene, xylene or a terpene, terpenoid or isoprenoid such as camphene or limonene; haloalkanes, such as dichloromethane, chloroform, carbon tetrachloride,

methvl bromide or a Fr on; esters, such as ethyl or propyl acetate, butyl formate or propyl or isopropyl butyrate or isobutyrate; and appropriate ethers and other lipophilic solvents. Solvents such as camphene
5 are of advantage in that they are biotolerated, so that it is not necessary to remove all solvent residues from the contrast agent prior to administration. Such high-melting solvents may also be advantageous in processes in which the emulsion is frozen and lyophilised, since
10 they will rapidly solidify under these conditions and so may enhance the structural integrity of the resulting microparticulate contrast agent.

As noted above, the emulsifying procedure is preferably effected in the presence of a surfactant.
15 Such emulsifying agents and/or any other additives, e.g. as hereinbefore described, may conveniently be predissolved in the aqueous phase.

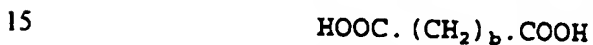
Prior to phase removal it may be advantageous to subject the emulsion to filtration and/or extrusion,
20 e.g. through a nozzle or one or more membranes of appropriate pore size, in order to enhance the uniformity of the size distribution of the microparticles and/or microballoons which are ultimately obtained.

25 The contrast agents of the invention may be stored and transported in dry form, in which condition they will normally be stable for long periods, being mixed with an appropriate liquid carrier (e.g. sterile water for injection, physiological saline or phosphate buffer)
30 prior to administration. In this way the concentration of the injected or otherwise administered contrast agent may be varied at will, depending on the precise nature of the application. They may also be stored in suspension in such carriers, being substantially
35 completely stable in aqueous media in the absence of esterase enzymes.

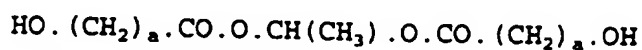
Polymers comprising repeating units of formula (II)

as hereinbefore defined are themselves novel products and constitute a further feature of the invention. As well as being useful starting materials for preparing contrast agents according to the invention, such
5 polymers may be of use as or in, for example, surgical implants such as sutures, soft tissue prostheses, sponges, films (e.g. artificial skin), wound dressings (e.g. hydrogel sheets), flexible sheet materials and articles such as containers formed therefrom, delayed
10 release formulations for drugs and agricultural chemicals, particulate imaging agents or plasticisers.

The novel polymers may be prepared by any convenient method, for example by reaction of a reactive derivative such as a dihalide of a diacid of formula



(where b is as hereinbefore defined) with a diol of formula



(where a is as hereinbefore defined), e.g. in an
20 appropriate organic solvent. The diol may itself be prepared by reacting an ethylidene halide such as the iodide with two moles of ω -hydroxyacid $\text{HO} \cdot (\text{CH}_2)_a \cdot \text{COOH}$,
e.g. in the presence of a base.

The following non-limitative Examples serve to
25 illustrate the invention.

EXAMPLE 1 - Preparation of intermediatesa) Ethylidene bis(16-hydroxyhexadecanoate)

5 1,8-Diazabicyclo [5.4.0]undec-7-ene (1,5-5) (DBU) (2.74 g, 0.018 mol) was added to 16-hydroxyhexadecanoic acid (4.90 g, 0.018 mol) in dimethylformamide (150 ml). After 5 minutes with stirring, ethylidene iodide (2.54 g, 0.009 mol) was added and the mixture was left with
10 stirring at 40°C for 3 days. The reaction mixture was cooled to 20°C and when precipitation was complete (2 hours) the precipitated monomer was isolated by filtration. The monomer was treated with activated carbon and recrystallised twice from dichloromethane to
15 give 1.03 g (20%) of the title product. Differential scanning calorimetry (DSC) indicated that onset melting temperature was 88.93°C. ¹H NMR (200 MHz, CDCl₃): δ 1.25 (s, 44H, CH₂), 1.45 (d, 3H, CH₃CH), 1.56 (m, 8H, CH₂), 2.30 (t, 4H, CH₂CO), 3.63 (t, 4H, 2 X CH₂O), 6.86 (q, 1H, CHCH₃). ¹³C NMR (50 MHz, CDCl₃): δ 20.86, 25.91, 26.98, 30.22, 30.44, 30.67, 30.84, 34.00, 35.30, 64.00, 89.00, 171.77 (C=O).

b) Ethylidene bis[16-(5-chlorocarbonylpentanoyloxy)-hexadecanoate]

25 In a three-necked round bottomed flask equipped with a reflux condenser, a glass gas inlet tube and a pressure equalizing dropping funnel was placed freshly distilled
30 adipoyl chloride (2.60 ml, 17.50 mmol) dissolved in absolute chloroform (15 ml). The temperature was raised to ca. 50°C and under a gentle stream of nitrogen through the solution, a solution of ethylidene bis(16-hydroxy-hexadecanoate) (1.0g, 1.75 mmol) in absolute chloroform
35 (30 ml) was added dropwise and left at this temperature a further 3 hours after addition. The mixture was then cooled to room temperature and quickly transferred into

a 50 ml round bottomed flask equipped for distillation under reduced pressure. Chloroform was first distilled off at normal pressure, then oil-pump vacuum was established and excess adipoyl chloride distilled off at
5 ca. 75°C, 5 mbar pressure, leaving the residual title compound (1.56g).

c) 16-Hexadecanoyloxyhexadecanoic acid

10 16-Hydroxyhexadecanoic acid (5.43g, 19.9 mmol) was dissolved in tetrahydrofuran (190 ml) and pyridine (2.36g, 29.9 mmol) was added. Palmitoyl chloride (5.48g, 19.9 mmol) was dissolved in tetrahydrofuran (10 ml) and added dropwise at room temperature. After
15 stirring at room temperature for 16 hours, the mixture was filtered and the filtrate evaporated under reduced pressure. The residue was dissolved in chloroform, washed with water (3 x 50 ml), and the organic phase was dried (MgSO₄). After evaporating under reduced pressure,
20 the residue was purified on a silica column, eluting with chloroform with increasing methanol concentration (from 1% to 2% methanol in chloroform) to give 8.41g (83%) of the title compound. ¹H-NMR (300-MHz, CDCl₃): δ 0.85 (t, 3H, CH₃), 1.20-1.35 (s, 46H, -CH₂-), 1.55-1.70 (m, 6H, -CH₂-), 2.25 (t, 2H, -CH₂-C(O)-O), 2.45 (t, 2H, -CH₂-COOH), 4.05 (t, 2H, -O-CH₂). ¹³C NMR (75 MHz, CDCl₃): δ 14.01, 22.57, 24.10, 24.91, 25.82, 28.53, 28.75, 28.94, 29.08, 29.15, 29.25, 29.36, 29.54, 31.81, 34.29, 35.16, 64.27, 76.48, 76.90, 77.10, 77.32, 169.50,
30 173.91.

d) 16-Hexadecanoyloxyhexadecanoyl chloride

16-Hexadecanoyloxyhexadecanoic acid (7.73g, 15.13 mmol)
35 prepared as in (c) above was dissolved in tetrahydrofuran (140 ml) and oxalyl chloride (4.80g, 37.83 mmol) was added dropwise. The mixture was stirred

- 12 -

at room temperature for 3 days and then the solvent and unreacted oxalyl chloride were evaporated under reduced pressure to give 8.0g (100%) of the title compound.

- 5 e) 1-[16-(16-Hexadecanoyloxyhexadecanoyloxy)-hexadecanoyloxy]ethyl 16-hydroxyhexadecanoate

Ethylidene bis(16-hydroxyhexadecanoate) (4.38g, 7.67 mmol) was dissolved in tetrahydrofuran (80 ml) and pyridine (0.61g, 7.71 mmol) was added. 16-Hexadecanoyl-
10 oxyhexadecanoyl chloride (4.18g, 7.90 mmol) was dissolved in tetrahydrofuran (20 ml) and added dropwise. After 3 days at room temperature the mixture was filtered and the filtrate was left at -20°C for 2 hours.
15 The precipitated product was filtered and purified by flash chromatography (silicagel, chloroform) to give 2.4g (29%) of the title compound. ¹H NMR (300 MHz, CDCl₃): δ 0.85 (t, 3H, CH₃), 1.2-1.4 (s, 90H, -CH₂-), 1.45 (d, 3H, -O-CH(CH₃)-O-), 1.5-1.7 (m, 14H, -CH₂-),
20 2.25 (m, 8H, -CH₂-C(O)-O-), 3.60 (t, 2H, -CH₂-OH), 4.05 (t, 4H, -C(O)-O-CH₂-), 6.85 (q, 1H, -O-CH(CH₃)-O-). ¹³C NMR (75 MHz, CDCl₃): δ 13.7, 19.1, 22.2, 24.2, 24.6, 25.2, 25.5, 28.2, 28.5, 28.7, 28.8, 29.0, 29.2, 31.5, 32.3, 33.7, 34.0, 62.5, 64.0, 88.0,
25 171.5, 173.5.

- f) Preparation of Methoxy-endcapped polyethylene glycols (PEGs)

30 Preparation of a Typical Polymer (MeO-PEG 2000)

An initiator solution was prepared by careful addition of potassium metal (0.400 g, 10.23 mmol) to methanol (1.300g, 40.57 mmol) in an inert atmosphere. A portion
35 of this initiator solution (0.220g, 1.32 mmol potassium methoxide) was injected into an ampoule containing ethylene oxide (10.000g, 227.00 mmol). The sealed

ampoule was allowed to stand at room temperature overnight. The temperature was then raised to 60°C and reaction allowed for 72 hours. After removal of unreacted monomer, the contents of the ampoule were
5 dissolved in dichloromethane and the solution neutralised with dilute aqueous hydrochloric acid. The polymer solution was washed three times with distilled water, rotary evaporated and then vacuum dried. Assignments for MeO-PEG polymers. ¹H-NMR: δ 2.7 (OH),
10 3.2 (OCH₃), 3.5 (-CH₂- main chain), 3.4 (-CH₂OCH₃). ¹³NMR: δ 58.5 (-OCH₃), 61.2 (-CH₂OH), 70.5 (-CH₂- main chain), 71.3 (-CH₂OCH₃), 72.2 (-CH₂CH₂OH). The GPC was recorded in THF and the molecular weight calibration was via PEG standards. GPC data for a typical sample: Mp:
15 2679, Mn: 2012, Mw: 2283. Polydispersity: 1.135.

g) General procedure for methoxy PEG chloroformate

PEG 2000 monomethyl ether (6.00g, 3.00 mmol) was
20 dissolved in toluene (50 ml) and dried by refluxing in a Dean Stark apparatus. Pyridine (0.24g, 3.00 mmol) was added at room temperature. Trichloromethyl chloroformate ("diphosgene") (0.60g, 3.00 mmol) was
25 dissolved in toluene (10 ml) and added dropwise. The mixture was stirred at room temperature for 12 hours and filtered. The solvent was evaporated under reduced pressure to give the title compound in quantitative yield.

30

EXAMPLE 2 - Preparation of polymers and emulsifiers

a) Polymer from ethylidene bis(16-hydroxyhexadecanoate) and adipoyl chloride

35

A solution of adipoyl chloride (0.48 g, 2.6 mmol) in xylene/trichloroethylene (80:20 v/v, 5ml) was added to a

- 14 -

solution of ethylidene bis(16-hydroxyhexadecanoate) (1.48 g, 2.6 mmol) from Example 1(a) above in xylene/trichloroethylene (80:20 v/v, 100 ml) at 60°C. After 2 days at 60°C under reduced pressure (147 mbar), the reaction mixture was cooled to 20°C. The solvent was evaporated under reduced pressure, the resulting polymer was dissolved in chloroform, reprecipitated in hexane and filtered, giving 1.05 g (60%) of the title compound as a white powder. Size Exclusion Chromatography (SEC): Mw=39068, Mn=9442, Mp=48536, Mw/Mn=4.138 (using polystyrene as standards). Differential scanning calorimetry (DSC) indicated that onset melting temperature was 48.61°C. ¹H NMR (200 MHz, CDCl₃): δ 1.28 (s, 44H, CH₂), 1.45 (d, 3H, CH₃CH), 1.62 (m, 12H, CH₂), 2.32 (m, 8H, CH₂CO), 4.02 (t, 4H, 2 X CH₂O), 6.88 (q, 1H, CHCH₃). ¹³C NMR (50MHz, CDCl₃): δ 20.85, 25.64, 25.68, 25.89, 27.16, 29.84, 30.15, 30.21, 30.44, 30.81, 35.08, 35.12, 35.27, 65.45, 88.98, 171.77 (C=O), 173.41 (C=O).

b) Random chain-extended polymer of PEG 1500, adipoyl chloride and ethylidene bis(16-hydroxyhexadecanoate) (0.37:1.85:1.75), multiblock.

To a suspension of ethylidene bis(16-hydroxyhexadecanoate) (1.0g, 1.75 mmol) in dimethoxyethane (10 ml) at room temperature was added freshly distilled adipoyl chloride (270 µl, 1.85 mmol). The temperature of the mixture was gradually raised to 60°C, and a colourless solution obtained. After 5 hours at this temperature PEG 1500 (0.55g, 0.37 mmol) was added and heating continued for a further 17 hours before the mixture was cooled to room temperature, the solvent evaporated and the solid residue stirred in petroleum ether (bp 40-60°C) for 15 minutes and filtered to give the title compound (1.30g) as a white solid.

- 15 -

c) Extended polymer from PEG 1500 and ethylidene bis[16-(5-chlorocarbonylpentanoyloxy)-hexadecanoate] (A-B-A)

5 Ethylidene bis[16-(5-chlorocarbonylpentanoyloxy)-hexadecanoate] prepared as in Example 1(b) (0.88 g, 1.02 mmol) was dissolved in toluene (15 ml) in a 100 ml 3-necked round bottomed flask equipped with a glass gas inlet tube and a reflux condenser. PEG 1500 (3.06g, 2.04 mmol) was added and the mixture heated at 60°C for 22 hours, cooled to room temperature and the solvent removed under reduced pressure to give the title compound (4.12g) as a white wax.

15 d) Extended polymer from PEG 1500 and ethylidene bis[16-(5-chlorocarbonylpentanoyloxy)hexadecanoate] (multiblock)

20 The reaction was performed as in Example 2(c), but with ethylidene bis[16-(5-chlorocarbonylpentanoyloxy)-hexadecanoate (1.02g, 1.18 mmol) in toluene (20 ml) and PEG 1500 (1.77g, 1.18 mmol) to give the title compound (2.29g) as a white wax.

25 e-h) Extended polymer of PEG, adipic acid and ethylidene bis(16-hydroxyhexadecanoate) (random multiblock)

30 A solution of PEG of appropriate molecular weight (A) (2.07 mmol) in the stated solvent (26 ml) was added via a syringe to a round bottomed flask containing ethylidene bis(16-hydroxyhexadecanoate) (B) (118 mg, 0.207 mmol), under nitrogen atmosphere. The resulting mixture was heated to 60°C, and when a clear solution had been obtained, adipoyl chloride (C) (417 mg, 2.277 mmol) was added via a syringe. The pressure was reduced to 250 mbar and the solution was stirred at 60°C for the

- 16 -

stated period. Remaining hydrogen chloride, evolved in the reaction, and the solvent were removed on a rotatory evaporator at reduced pressure and 60°C for 3 hours, and subsequently under vacuum (<0.1mm Hg) at 60°C for 24 hours. Finally, the polymer was precipitated from an acetone solution by adding petroleum ether, and cooling in an ice bath for 2 hours. Filtration yielded 3.5g of the polymer as a white waxy solid.

In total four different block copolymers differing in the molecular weight of the starting PEGs were prepared by this method; the conditions specific for each polymerisation are given in Table 1 below. ¹³C NMR- and ¹H NMR-spectra of the polymers was in agreement with the expected products.

Table 1

Entry	Mw for starting PEG	Molar ratio A:B:C ¹	Solvent	Reaction time (hours)
e	400	10:1:11	Diglyme-xylene	21
f	600	10:1:11	Diglyme	24
g	1500	10:1:11	Dimethoxyethane	21
h	2000	10:1:11	1,1,2-Tri-chloroethylene	92

¹) The letters refers to the reactants as specified in the text above.

i) PEG 2300 methyl ether 16-hexadecanoyloxyhexadecanoate

PEG 2300 methyl ether (10.000g, 4.35 mmol) was dissolved in tetrahydrofuran (90 ml) and pyridine (0.413g, 5.22 mmol) was added. 16-Hexadecanoyloxyhexadecanoyl

- 17 -

chloride (2.301g, 4.35 mmol) was dissolved in tetrahydrofuran (10 ml) and added dropwise. After stirring for 3 days at room temperature, the mixture was filtered and the solvent was evaporated under reduced pressure. The residue (12.08g) was purified on a silica column, eluting with chloroform with increasing methanol concentration (from 1% to 3% methanol in chloroform) to give 5.20g (43%) of the title compound. ^1H NMR (300 MHz, CDCl_3): δ 0.80-0.87 (m, CH_3), 1.21 (s, (br), CH_2), 1.53-1.62 (m, CH_2), 2.20-2.35 (m, CH_2CO), 3.34 (s, CH_3O), 3.61 (s, $\text{OCH}_2\text{CH}_2\text{O}$), 4.02 (t, $\text{COOCH}_2\text{CH}_2\text{O}$), 4.19 (t, $\text{COOCH}_2\text{CH}_2\text{O}$). ^{13}C NMR (75 MHz, CDCl_3): δ 13.95, 22.49, 24.71, 24.83, 25.74, 28.45, 28.95, 29.07, 29.16, 29.28, 29.34, 29.40, 29.46, 31.72, 34.05, 34.21, 58.85, 63.15, 64.19, 69.01, 70.37, 71.73, 173.64, 173.82.

j) PEG 5000 methyl ether 16-hexadecanoyloxy-hexadecanoate

PEG 5000 methyl ether (7.500g, 1.50 mmol) was dissolved in toluene (90 ml) and dried by refluxing in a Dean Stark apparatus. Pyridine (0.143g, 1.80 mmol) was added followed by addition (dropwise) of 16-hexadecanoyloxy-hexadecanoyl chloride (1.191g, 2.25 mmol) dissolved in toluene (10 ml). The mixture was heated to reflux and after stirring under reflux for 3 days the mixture was cooled to room temperature and precipitated into hexane. After filtering, the precipitate was washed with hexane and dried (MgSO_4). After evaporation under reduced pressure, the residue was purified on a silica column, eluting with chloroform with increasing methanol concentration (from 1% to 3% methanol in chloroform) to give 5.93g (72%) of the title compound. ^1H NMR (300 MHz, CDCl_3): δ 0.82-0.86 (m, CH_3), 1.22 (s, (br), CH_2), 1.53-1.62 (m, CH_2), 2.20-2.35 (m, CH_2CO), 3.34 (s, CH_3O), 3.61 (s, $\text{OCH}_2\text{CH}_2\text{O}$), 4.01 (t, $\text{COOCH}_2\text{CH}_2\text{O}$), 4.18 (t, COOCH_2O). ^{13}C NMR (75 MHz, CDCl_3): δ 13.66, 22.21, 24.43, 24.54,

25.46, 28.17, 28.67, 28.79, 28.87, 28.99, 29.06, 29.11,
29.17, 31.44, 33.73, 33.93, 58.57, 62.87, 63.90, 68.72,
69.62, 69.86, 70.09, 71.45, 76.85, 173.35, 173.53.

5 k) PEG 10000 methyl ether 16-hexadecanoyloxyhexa-
decanoate

PEG 10000 methyl ether (7.500g, 0.75 mmol) was dissolved
in toluene (140 ml) and pyridine (0.107g, 1.35 mmol) was
10 added. The solution was heated to 60°C and 16-
hexadecanoyloxyhexadecanoyl chloride (0.595g, 1.12 mmol)
dissolved in toluene (10 ml) was added dropwise. The
mixture was heated to reflux and after stirring under
reflux for 3 days the mixture was cooled to room
15 temperature and precipitated into hexane. After
filtering, the precipitate was washed with hexane and
dried. Flash chromatography on a silica column, eluting
with 5% methanol in chloroform, gave 5.39g (68%) of the
title compound. ¹H NMR (300 MHz, CDCl₃): δ 0.84 (t,
20 CH₃), 1.21 (s, (br), CH₂), 1.55-1.60 (m, CH₂), 2.20-2.35
(m, CH₂CO), 3.34 (s, CH₃O), 3.61 (s, OCH₂CH₂O), 4.01 (t,
COOCH₂CH₂O), 4.18 (t, COOCH₂CH₂O). ¹³C NMR (75 MHz,
CDCl₃): δ 13.94, 22.48, 24.70, 24.82, 25.73, 28.94,
29.05, 29.14, 29.26, 29.33, 29.39, 29.45, 31.71, 34.00,
25 58.84, 63.14, 68.99, 69.36, 69.86, 69.97, 70.01, 70.36,
70.74, 70.82, 70.86, 71.72, 77.10, 173.62, 173.80.

26 l) 16-[ω-Methoxy-PEG 2000-carbonyloxy]hexadecanoic
acid 1-[16-(16-hexadecanoyloxyhexadecanoyloxy)-
30 hexadecanoyloxy]ethyl ester

Methoxy PEG 2000 chloroformate (1.90g, 0.95 mmol) was
dissolved in toluene (90 ml), and pyridine (0.09g, 1.13
mmol) was added. 1[[16-(16-Hexadecanoyloxy-
35 hexadecanoyloxy)hexadecanoyloxy]ethyl 16-hydroxyhexa-
decanoate (1.00g, 0.95 mmol) was dissolved in toluene
(10 ml) and added dropwise. The mixture was heated to

reflux and after stirring under reflux for 10 hours, the mixture was cooled to room temperature and filtered.

The solvent was evaporated under reduced pressure. The residue was purified on a silica column using chloroform containing 2% methanol, to give 1.00g (35%) of the title compound.

¹H-NMR (300 MHz, CDCl₃): δ 0.85 (t, CH₃), 1.20-1.33 (m, CH₂), 1.45 (d, -O-CH(CH₃)-O), 1.5-1.7 (m, CH₂), 2.0 (H₂O), 2.2-2.3 (m, -CH₂-C(O)-O), 3.35 (s, CH₃-O-), 3.5-3.7 (s, -OCH₂CH₂O-), 4.03 (t, -C(O)-O-CH₂-), 4.10 (t, -CH₂-O-C(O)-O-), 4.26 (m, -O-C(O)-O-CH₂-CH₂-O-), 6.8-6.9 (q, -O-CH(CH₃)-O). ¹³C-NMR (75 MHz, CDCl₃): δ 13.7, 19.2, 22.1, 24.2, 24.6, 25.2, 25.5, 28.2-29.2, 31.5, 33.9, 34.0, 58.7, 64.0, 66.3, 67.9, 68.5, 70.0, 71.5, 87.9, 171.5, 173.7.

m) 16-[ω-Methoxy PEG 5000 carbonyloxy]hexadecanoic acid 1-[16-(16-hexadecanoyloxyhexadecanoyloxy)-hexadecanoyloxy]ethyl ester

Methoxy PEG 5000 chloroformate (8.50g, 1.70 mmol) was dissolved in toluene (90 ml) and pyridine (0.146g, 1.85 mmol) was added. 1-[16-(16-Hexadecanoyloxyhexadecanoyloxy)hexadecanoyloxy]ethyl 16-hydroxyhexadecanoate

(1.79g, 1.70 mmol) was dissolved in toluene (10 ml) and added dropwise. The mixture was heated to reflux and after stirring under reflux for 3 days the mixture was cooled to room temperature and filtered. The solvent was evaporated under reduced pressure and the residue was purified on a silica column, eluting with chloroform

with increasing methanol concentration (from 3% to 5% methanol in chloroform) to give 3.90g (38%) of the title compound.

¹H-NMR (300 MHz, CDCl₃): δ 0.85 (t, CH₃), 1.20-1.33 (m, CH₂), 1.45 (d, -O-CH(CH₃)-O), 1.5-1.7 (m, CH₂), 1.8 (H₂O), 2.2-2.3 (m, -CH₂-C(O)-O), 3.35 (s, CH₃-O-), 3.5-3.7 (s, -OCH₂CH₂O-), 4.03 (t, -C(O)-O-CH₂-), 4.10 (t, -CH₂-O-C(O)-O-), 4.26 (m, -O-C(O)-O-CH₂-CH₂-O-), 6.8-6.9 (q, -O-CH(CH₃)-O).

- 20 -

n) 16-[ω -Methoxy PEG 10000 carbonyloxy]hexadecanoic acid 1-[16-(16-hexadecanoyloxyhexadecanoyloxy)hexadecanoyloxy]ethyl ester

5 Methoxy PEG 10000 chloroformate (7.50g, 0.75 mmol) was dissolved in toluene (90 ml), and pyridine (0.063g, 0.80 mmol) was added. 1-[16-(16-Hexadecanoyloxyhexadecanoyloxy)hexadecanoyloxy]ethyl 16-hydroxyhexadecanoate
10 (0.79g, 0.75 mmol) was dissolved in toluene (10 ml) and added dropwise. The mixture was heated to reflux and after stirring under reflux for 3 days the mixture was cooled to room temperature and filtered. The solvent was evaporated off under reduced pressure. The residue was purified on a silica column, eluting with chloroform
15 with increasing methanol concentration (from 3% to 5% methanol in chloroform) to give 1.60g (19%) of the title compound. $^1\text{H-NMR}$ (300 MHz, CDCl_3): δ 0.85 (t, CH_3), 1.20-1.33 (m, CH_2), 1.45 (d, $-\text{O}-\text{CH}(\text{CH}_3)-\text{O}-$), 1.5-1.7 (m, CH_2), 2.2-2.3 (m, $-\text{CH}_2-\text{C}(\text{O})-\text{O}-$), 3.35 (s, $\text{CH}_3-\text{O}-$), 3.5-3.7 (s, $-\text{OCH}_2\text{CH}_2\text{O}-$), 4.03 (t, $-\text{C}(\text{O})-\text{O}-\text{CH}_2$), 4.10 (t, $-\text{CH}_2-\text{O}-\text{C}(\text{O})-\text{O}-$), 4.26 (m, $-\text{O}-\text{C}(\text{O})-\text{O}-\text{CH}_2-\text{CH}_2-\text{O}-$), 6.8-6.9 (q, $-\text{O}-\text{CH}(\text{CH}_3)-\text{O}-$).

25 EXAMPLE 3 - Preparation of polymer particles

a) Particles from polymer made from ethylidene bis(16-hydroxyhexadecanoate) and adipoyl chloride

30 10 ml of a 5% w/v solution of the polymer from Example 2(a) in toluene was added to 30 ml of a 5 wt% solution of human serum albumin in water. The two phases were mixed with an Ultra Turax® T25 mixer at 20,000 rpm for 1 minute, frozen on a dry ice/methanol bath, and
35 lyophilized for 18 hours, giving a slightly yellow powder. Light microscopy indicated formation of microparticles.

- b) Particles from polymer made from ethylidene bis(16-hydroxyhexadecanoate) and adipoyl chloride

5 10 ml of a 10% w/v solution of the polymer from Example 2(a) in p-xylene was added to 30 ml of a 5 wt% solution of human serum albumin in water. The mixture was mixed with an Ultra Turax® T25 mixer at 20,000 rpm for 1 minute and 30 seconds, frozen on a dry ice/methanol bath, and lyophilized for 18 hours, giving a white powder. Light microscopy indicated formation of microparticles.

- c) Particles from polymer made from ethylidene bis(16-hydroxyhexadecanoate) and adipoyl chloride

15 10 ml of a 5% w/v solution of the polymer from Example 2(a) in p-xylene was added to 30 ml of a 5 wt% solution of modified starch (Lyckeby, Sweden, PU-24.000) in water. The mixture was mixed with an Ultra Turax® T25 mixer at 20,000 rpm for 1 minute and 30 seconds, frozen on a dry ice/methanol bath, and lyophilized for 18 hours, giving a white powder. Light microscopy indicated formation of microparticles.

- 25 d) Particles from polymer made from ethylidene bis(16-hydroxyhexadecanoate) and adipoyl chloride

30 10 ml of a 5% w/v solution of the polymer from Example 2(a) in p-xylene was added to 30 ml of a 0.8 wt% solution of polyvinyl alcohol in water. The mixture was mixed with an Ultra Turax® T25 mixer at 20,000 rpm for 1 minute, frozen on a dry ice/ methanol bath, and lyophilized for 18 hours, giving a white powder. Light microscopy indicated formation of microparticles.

- 22 -

e) Particles from polymer made from ethylidene bis(16-hydroxyhexadecanoate) and adipoyl chloride

10 ml of a 5% w/v solution of the polymer from Example
5 2(a) in p-xylene was added to 30 ml of a 1 wt% solution
of gelatin in water. The mixture was mixed with an
Ultra Turax® T25 mixer at 20,000 rpm for 1 minute,
frozen on a dry ice/methanol bath, and lyophilized for
18 hours, giving a white powder. Light microscopy
10 indicated formation of microparticles.

f) Particles from polymer made from ethylidene bis(16-hydroxyhexadecanoate) and adipoyl chloride

15 5 ml of a 5% w/v solution of the polymer from Example
2(a) in (-)-camphene maintained at 60°C was added to 15
ml of a 5 wt% solution of human serum albumin in water
at the same temperature. The mixture was mixed hot with
an Ultra Turax® T25 mixer at 20,000 rpm for 1 minute,
20 frozen on a dry ice/methanol bath, and lyophilized for
48 hours, giving a white powder. Light microscopy
indicated formation of microparticles.

g-n) Particles from polymer made from ethylidene bis(16-hydroxyhexadecanoate) and adipoyl chloride,
25 stabilized in dispersion with block copolymer

General description

30 10 ml of a 5% w/v solution of the polymer from Example
2(a) in (-)-camphene maintained at 60°C was added to 30
ml of an aqueous solution of block copolymer from
Example 2 above (see Table 2) at the same temperature
and with concentrations as given in Table 2. The
35 mixture was mixed with a rotor-stator mixer (Ultra
Turax® T25) at slow speed for several minutes, frozen on
a dry ice/ methanol bath, and lyophilized for 48 hours,

giving a white powder.

Table 2

Example 3	Block copolymer from Example 2	Conc. [% w/w]
g	2d	1
h	2d	2
i	2h	1
j	2h	2
k	2j	1
l	2k	1
m	2l	2
n	2n	2

o) Particles from polymer made from ethylidene bis(16-hydroxyhexadecanoate) and adipoyl chloride

16ml of a 3% w/v solution of the polymer from Example 2(a) in (-)-camphene maintained at 70°C was added to 64 ml of an aqueous solution containing 1% w/v of the block copolymer from Example 2(k) and 5% w/v of PEG 3000 at the same temperature. The mixture was mixed with a rotor-stator mixer at moderate speed for up to 5 minutes, frozen on a dry ice/methanol bath, and lyophilized for 48 hours, giving a white powder. The dry product was dispersed in saline solution on a laboratory shaker for 16 hours at a concentration of 10 mg dry material/ml.

p) Particles from polymer made from ethylidene bis(16-hydroxyhexadecanoate) and adipoyl chloride

The procedure of Example 3(o) was repeated, but with

- 24 -

cyclooctane in place of (-)-camphene as organic solvent.

q) Particles from polymer made from ethylidene bis(16-hydroxyhexadecanoate) and adipoyl chloride

5

The procedure of Example 3(o) was repeated, but with cyclohexane in place of (-)-camphene as organic solvent.

10

r) Particles from polymer made from ethylidene bis(16-hydroxyhexadecanoate) and adipoyl chloride

15

The procedure of Example 3(o) was repeated, except that emulsification was carried out at 60°C using 28 ml of a 7.5% w/v solution of the polymer from Example 2(a) in (-)-camphene and 62 ml of an aqueous solution containing 2% w/v of the polymer from Example 2(k).

EXAMPLE 4 - Acoustic characterizations.

General procedure

20

Dry powders of polymer particles prepared according to Example 3 above were redispersed to 10 mg/ml dry material in MilliQ water by shaking on a laboratory shaker for 12-16 hours. Examination by light microscopy indicated formation of particle dispersions. The particles floated readily, as expected for gas-containing particles.

25

Acoustic effects in vitro

30

The acoustic effect of suspensions prepared as above was obtained by measuring the ultrasonic transmission through solutions of different concentrations (mg/ml) in an aqueous carrier liquid, using a 3.5 MHz broadband transducer in a pulse-reflection technique. The aqueous carrier liquid was used as reference, and measurements were performed on serial dilutions with the carrier

35

- 25 -

liquid until the signal was reduced to approximately 3-5 db/cm. The concentration necessary to give an attenuation of 8 db/cm was noted (Table 3); hence low values indicate a good contrast effect. The obtained
 5 acoustic effects are at a level indicating that the products can be expected to be useful as ultrasound contrast agents. According to theoretical considerations, solid (as opposite to gas-containing) particles of the same size and at the same dilutions
 10 should give an acoustic attenuation of less than 0.1 db/cm.

Table 3

15 Acoustic measurements of particles from Example 3 above. The acoustic measurements are given in column 3 as the concentration giving a contrast effect of 8 db/cm, i.e. half value of saturated signal. At higher concentrations, the signal intensity increased until
 20 saturation was observed.

Example 4	Particles of Example	Particle conc. at 8 db/cm [mg/ml]
a	3a	1.0
25 b	3b	0.12
c	3c	0.03
d	3d	0.16
e	3e	0.35
f	3f	0.15
30 g	3g	0.04
h	3h	0.02
i	3i	0.02
j	3j	0.02

- 26 -

k	3k	0.03
l	3l	0.01
m	3m	0.09
n	3n	0.08

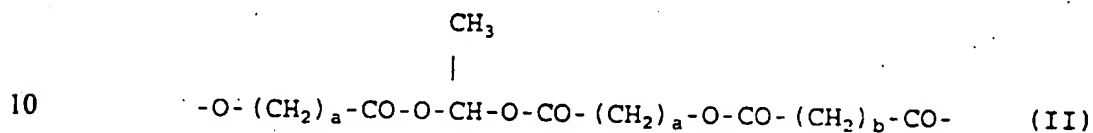
5

EXAMPLE 5 - In vivo characterizations

10 The powders of polymer particles prepared as described
in Example 3(a)-(f) above were redispersed in sterile
0.9% (w/v) NaCl (aq) solution by shaking on a laboratory
shaker for 12-16 hours. The dispersions were injected
in leg veins of dogs, and short axis transthoracic
15 echocardiatic images were obtained using a Vingmed Sound
CFM750 ultrasonic scanner at 5 MHz. The image sequences
were stored on video. The particle dispersions all
caused very sharp contrast enhancement in both
ventricles and also caused significant myocardial
contrast enhancement (MCE), visible on live video
20 sequences. MCE was demonstrated in the anterior and
posterior walls. The duration of contrast effect
reveals that the particle dispersions circulated in vivo
for several minutes after injection. The presence of
myocardial contrast and the long duration of contrast
25 indicates that the in vivo stability is very good.

CLAIMS

1. A contrast agent comprising gas-containing polymer microparticles and/or microballoons
 5 characterised in that the polymer is a biodegradable polymer comprising repeating units of formula (II)



(where a represents an integer in the range 9-19 and b represents an integer in the range 1-8).

- 15 2. A contrast agent as claimed in claim 1 wherein a represents an integer in the range 13-17 and b represents an integer in the range 3-6.

- 20 3. A contrast agent as claimed in claim 1 wherein a is 15 and b is 4.

4. A contrast agent as claimed in any of claims 1 to 3 incorporating one or more additives selected from
 emulsifying agents, coating agents, plasticisers,
 25 bulking agents, cryoprotectants and antioxidants.

5. A contrast agent as claimed in claim 4 incorporating an emulsifying agent selected from fatty acids, carbohydrate esters of fatty acids, triglyceride
 30 esters of fatty acids, proteins, phospholipids, polysaccharides and surface active polymers.

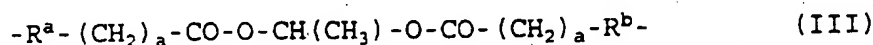
6. A contrast agent as claimed in claim 5 wherein the emulsifying agent is human serum albumin, modified
 35 starch or gelatin.

7. A contrast agent as claimed in claim 5 wherein

- 28 -

the emulsifying agent is polyvinyl alcohol or a block copolymer/extended polymer.

8. A contrast agent as claimed in claim 7 wherein
5 the block copolymer/extended polymer contains
polyethylene glycol units as the hydrophilic blocks and
units of formula (III)



10

(where a is as defined in claim 1 and R^a and R^b each
represent valence bonds or linking groups) in the
hydrophobic part as an extending moiety or as a moiety
in an oligomeric or polymeric block.

15

9. A contrast agent as claimed in claim 8 wherein
 R^a and R^b are each selected from carbonyl groups and
groups of formula $-O-CO-(CH_2)_b-CO-O-$ (where b is as
defined in claim 1).

20

10. A contrast agent as claimed in claim 7 wherein
the emulsifying agent is an extended polymer comprising
a methoxy-terminated polyethylene glycol hydrophilic
block acylated with a hydrophobic moiety comprising a
25 chain of two or more fatty acids.

25

11. A contrast agent as claimed in claim 10
wherein the hydrophobic moiety is an acyloxyacyl group.

30

12. A contrast agent as claimed in claim 11
wherein the hydrophobic moiety is 16-hexadecanoyloxy-
hexadecanoyl.

35

13. Use of a contrast agent as claimed in any of
claims 1 to 12 in diagnostic imaging.

14. Use of a contrast agent as claimed in any one

of claims 1 to 12 in diagnostic ultrasonic imaging.

15. Use of a contrast agent as claimed in any of claims 1 to 12 in magnetic resonance imaging.

5

16. A method of generating enhanced images of a human or non-human animal body which comprises administering to said body a contrast agent as claimed in any of claims 1 to 12 and generating an ultrasound or magnetic resonance image of at least a part of said body.

10

17. A process for the preparation of a contrast agent as claimed in claim 1 which comprises incorporation of a gas into a biodegradable polymer comprising repeating units of formula (II) as defined in claim 1 so as to form polymer microparticles and/or microballoons.

15

18. A process as claimed in claim 17 which comprises emulsifying a solution of the polymer in a water-immiscible organic solvent in an aqueous phase and thereafter removing at least the organic phase under an atmosphere of the gas which is to be incorporated.

20

25

19. A process as claimed in claim 18 wherein the aqueous phase contains an emulsifying agent as defined in any of claims 5 to 12.

30

20. A process as defined in claim 18 or claim 19 wherein the emulsion is filtered and/or extruded prior to phase removal.

21. Biodegradable polymers comprising repeating units of formula (II) as defined in claim 1.

35

22. Biodegradable polymers as claimed in claim 21

- 30 -

wherein a represents an integer in the range 13-17 and b represents an integer in the range 3-6.

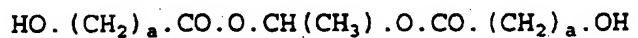
23. Biodegradeable polymers as claimed in claim 21
5 wherein a is 15 and b is 4.

24. A surgical implant, soft tissue prosthesis, sponge, film, wound dressing, flexible sheet, container, medical or agricultural delayed release formulation,
10 particulate imaging agent or plasticiser comprising a polymer as claimed in any of claims 21 to 23.

25. A process for the preparation of a polymer as claimed in claim 21 which comprises reacting a reactive
15 derivative of a diacid of formula



(where b is as defined in claim 1) with a diol of
20 formula



(where a is as defined in claim 1).
25

INTERNATIONAL SEARCH REPORT

Int. Application No
PCT/GB 95/02109

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A61K49/00 A61M31/00 A61F2/30 A61K9/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,93 17718 (NYCOMED IMAGING A S) 16 September 1993 cited in the application see page 18, line 17 - line 31; claims see page 19, line 38 - line 20 ---	1-25
P,X	WO,A,95 06518 (NYCOMED IMAGING A S ;DUGSTAD HARALD (NO); FOSS PER ANTONIUS (NO);) 9 March 1995 see page 7, line 1 - line 25; claims see page 18, line 23 - page 20, line 10; examples see page 15, line 6 - line 14-25 --- -/--	1-25

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

A document defining the general state of the art which is not considered to be of particular relevance

E earlier document but published on or after the international filing date

L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

O document referring to an oral disclosure, use, exhibition or other means

P document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

Z document member of the same patent family

Date of the actual completion of the international search

12 January 1996

Date of mailing of the international search report

19.01.1996

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl,
Fax (+ 31-70) 340-3016

Authorized officer

Berte, M

Internal Application No
PCT/GB 95/02109

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/GB95/02109

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim 16 is directed to a method of treatment of (diagnostic method practised on) the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

information on patent family members

Int. Application No

PCT/GB 95/02109

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9317718	16-09-93	BG-A- 99070	28-08-95
		BG-A- 99072	29-09-95
		CA-A- 2130670	07-09-93
		CN-A- 1084410	30-03-94
		CN-A- 1084524	30-03-94
		CZ-A- 9402148	12-04-95
		CZ-A- 9402152	16-08-95
		EP-A- 0629214	21-12-94
		EP-A- 0627942	14-12-94
		FI-A- 944077	05-09-94
		FI-A- 944078	05-09-94
		WO-A- 9318070	16-09-93
		HU-A- 69070	28-08-95
		HU-A- 68612	28-07-95
		JP-T- 7505177	08-06-95
		JP-T- 7505148	08-06-95
		LT-A,B 388	25-09-94
		LT-A,B 391	25-09-94
		NO-A- 943272	05-09-94
		NO-A- 943273	05-09-94
		SK-A- 105694	12-04-95
		ZA-A- 9301594	27-09-93
		ZA-A- 9301599	28-12-93
WO-A-9506518	09-03-95	AU-B- 7541594	22-03-95
WO-A-9521631	17-08-95	AU-B- 1668795	29-08-95
WO-A-9217212	15-10-92	AU-B- 1425392	02-11-92
		CA-A- 2107108	29-09-92
		EP-A- 0576519	05-01-94
		JP-T- 6507884	08-09-94
WO-A-9204392	19-03-92	AU-B- 662490	07-09-95
		AU-B- 8525691	30-03-92
		BG-A- 97499	24-03-94
		CA-A- 2091165	08-03-92
		EP-A- 0547126	23-06-93
		HU-A- 66370	28-11-94
		IL-A- 99438	31-07-95

INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No

PCT/GB 95/02109

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9204392		JP-T- 6503369	14-04-94
		LT-A- 958	27-03-95
		SK-A- 15793	06-10-93
		AU-B- 663866	26-10-95
		AU-B- 1443992	02-11-92
		CA-A- 2107106	29-09-92
		WO-A- 9217436	15-10-92
		EP-A- 0577659	12-01-94
		JP-T- 6508108	14-09-94
		NO-A- 933430	08-11-93

THIS PAGE BLANK (USPTO)